Journal of Chromatography A, 1218 (2011) 2127–2132

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Development of a solid-phase extraction with capillary liquid chromatography tandem mass spectrometry for analysis of estrogens in environmental water samples

Petr Kozlík[a], Zuzana Bosáková[a,*], Eva Tesařová[b], Pavel Coufal[a], Radomír Čabala[a]

[a] Department of Analytical Chemistry, Faculty of Science, Charles University in Prague, Albertov 2030, 128 40 Prague 2, Czech Republic
[b] Department of Physical and Macromolecular Chemistry, Faculty of Science, Charles University in Prague, Albertov 2030, 128 40 Prague 2, Czech Republic

ARTICLE INFO

Article history:
Received 30 July 2010
Received in revised form 3 October 2010
Accepted 6 October 2010
Available online 13 October 2010

Keywords:
Estrogen pollutants
Capillary liquid chromatography–tandem mass spectrometry
Solid-phase extraction
Environmental water sample
Quantitation

ABSTRACT

Capillary liquid chromatography (cLC) hyphenated with tandem mass spectrometry (MS–MS) was used to separate and quantitate trace concentrations of five estrogens in aqueous samples. New C_{18}-based sorption materials bound to the silica support by monomeric and polymeric mechanisms were compared and tested for solid-phase extraction (SPE) of selected analytes with respect to optimization of their preconcentration yield. Application of an endcapped, monomer-bound preconcentration Discovery DSC-18Lt column under the optimized conditions provides yields in the range from 95 to 100% with a high repeatability ($n = 3$, RSD $\leq 7.2\%$). Using the electrospray ionization in the positive mode (ESI+), the cLC–MS–MS system (the Zorbax SB C18 capillary column and a binary mobile phase of acetonitrile and water containing 0.1% formic acid in both the components) was optimized to attain a sufficient retention of the early eluting estriol, a satisfactory resolution of the analytes and the maximum sensitivity of the determination. Both the isocratic and gradient elution were used and the optimized gradient method permitted analyses of aqueous environmental samples in 14 min within a linearity range from 6.1 to 25.0 (LOQ of analytes) to 500 ng/L and with a very good linearity ($r > 0.9981$) for all the estrogens studied. The detection limits are in the range from 3.0 to 6.8 ng/L (1 μL injection volume). Six environmental water samples were analyzed and the studied estrogens were found in the Vltava river sample collected in Prague (13.2 ng/L for 17β-estradiol) and in the inlet to the wastewater treatment plant in Prague, at an overall concentration of 371.4 ng/L.

1. Introduction

The greatly varied human activities cause continuously increasing environmental pollution, with the pollutant range becoming progressively more complex. A relatively ubiquitous class of pollutants are substances which affect the endocrine systems of a wide spectrum of organisms. These substances, called endocrine disruptors (EDs), may imitate the activities of endogenous hormones, or may interfere with them [1,2]. Mostly, they enter the environment as a part of human wastes and could create a risk for reproduction of organisms even if they are present at very low concentrations. Changes in the sex and in the reproduction functions for reptiles, birds, amphibians, crustacea and fish caused by the presence of estrogens in the environment have been published [3]. During recent decades, the occurrence of testicular tumors and developmental defects of reproduction organs in the human population have increased and the quality of human spermiogram has substantially deteriorated [4,5]. The presence of estrogen pollutants, together with unhealthy lifestyle, may be one of the causes. Some negative effects of EDs on the human reproduction have already been demonstrated (diethylstilbestrol), or are being tested (e.g., bisphenol, A nonylphenol, etc.) [4–7].

Biogenic hormones, such as 17β-estradiol (βE2), 17α-estradiol (αE2), estriol (E3), estrone (E1) and the synthetic contraceptives mestranol (M) and 17α-ethynylestradiol (EE2), belong to the group of estrogen pollutants. After their absorption in the organism, they are transformed into more polar glucuronates or sulfates and removed by excretion. They enter wastewaters as a part of excrements and are treated in a wastewater treatment plant (WWTP) where they can be degraded by microorganisms or adsorbed on solid particles. They can then be liberated again during the subsequent treatment of the sewage or when using wastes as fertilizers [8–10].

The analytical methods for estrogens have already been reviewed [11–15]. Most analyses are directed toward the determination of free estrogens in aqueous matrices, such as surface

* Corresponding author at: Department of Analytical Chemistry, Charles University in Prague, Faculty of Science, Albertov 2030, 128 43, Prague 2, Czech Republic. Tel.: +420 221951231; fax: +420 224 913 538.
E-mail address: bosakova@natur.cuni.cz (Z. Bosáková).

0021-9673/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2010.10.033

water, WWTP outflow or urine [11]. However, these compounds have also been studied in solid samples, such as sediments or sewage sludge [16–19]. Recently, not only the free estrogens, but also their conjugates (glucuronates and sulfates) have been determined [20–22]. Owing to the low concentrations of estrogen pollutants (tens to hundreds of ng/L) in environmental samples, the analyses require highly sensitive methods combined with efficient preseparation and preconcentration techniques. Solid-phase extraction (SPE) using various sorbent types and elution solvents is one of the most often employed sample pretreatment methods [11,23]. However, it suffers from some principal and common drawbacks, involving poor selectivity, coextraction of interfering matrix components and lower recoveries (up to 90%). Application of immunoaffinity extraction of estrogens has also been published [11].

Immunochemical methods (ELISA), combined with gas chromatography (GC) and liquid chromatography (HPLC), are most often used for the estrogen determination. Each of these techniques has some advantages but also suffers from some drawbacks. For example, immunochemical methods permit rapid screening and a preconcentration step is sometimes unnecessary [24,25]. However, non-specific binding interactions may decrease the sensitivity of determination. Gas chromatography permits rapid and sensitive analyses, mostly in combination with mass- (GC–MS) or tandem mass spectrometric detection (GC–MS–MS), however, conversion of the analytes into more volatile derivatives, usually through silylation or acylation, is commonly required after the preconcentration step [13,26,27]. HPLC methods permit direct analyses of estrogens without preliminary derivatization and many ionization and monitoring modes are available for mass spectrometric detection. HPLC–MS and HPLC–MS–MS techniques are most common, followed by HPLC with UV photometric, fluorescence and electrochemical detection in analyses of aqueous samples of estrogen pollutants [28–32]. HPLC–MS–MS with electrospray ionization in the negative mode (ESI-) exhibits a very high detection sensitivity [12,29,32,33].

The problems of determination of estrogen pollutants in river and sewage waters have primarily been elaborated in Japan, Spain and Germany and only a few studies have been carried out about the river water pollution with estrogens in the Czech Republic, namely a GC-MS method for the estrogen analysis of the Vltava river sediments in Prague [18,19], a gas chromatography—time of flight mass spectrometric (GC-TOF-MS) method for the Vltava river water at several sites close to Prague [34] and a liquid chromatography—ion-trap mass spectrometric method for the Svratka river water at various sites [35].

The contemporary trends in analytical activities lead to efforts concerning the introduction of approaches gentle to the environment (the green chemistry), involving limited use of nonaqueous solvents and minimization of the consumption of solutions, sorbents and chemicals [36]. One way to attaining these requirements is miniaturization of the separation system, provided that sufficient sensitivity, selectivity, reliability and robustness of the determination are preserved. This is often difficult to attain. In addition to capillary electrophoresis (CE), micellar electrokinetic chromatography (MEKC) and capillary electrochromatography (CEC), a promising approach seems to be the use of capillary liquid chromatography (cLC), which preserves the separation advantages of classical HPLC, saves the chemicals and attains a sufficiently high sensitivity when combined with mass spectrometric detection [37].

This work aims at development of a new miniaturized cLC method applicable to identification and quantitation of selected estrogen pollutants in the environment, at very low analyte concentrations of tenths to hundreds of ng/L. A modern capillary liquid chromatograph hyphenated with the MS–MS detection and employing less frequent positive electrospray ionization (ESI) mode is used for this investigation. Additionally, a new type of sorbents is tested for solid-phase extraction which should permit effective preconcentration of estrogens and minimize its drawbacks mentioned above. The proposed cLC–ESI–MS–MS procedure is optimized with respect to the limits of detection (LOD) and limits of quantitation (LOQ), and the values obtained are evaluated and compared with the published ones.

2. Experimental

2.1. Materials and reagents

Acetonitrile and methanol (gradient grade) were supplied by Sigma–Aldrich (St. Louis, USA). Ethyl acetate and acetone (both of the p.a. purity) were provided by Penta (Chrudim, the Czech Republic). Formic acid (purity 98–100%) was purchased from Merck (Damstadt, Germany). Standards of estrone (> 99%), 17α-estradiol (99%), 17β-estradiol (98%), 17α-ethynylestradiol (98%) and estriol (99%) were purchased from Sigma–Aldrich (St. Louis, USA); for their structures, see Fig. 1. Stock solutions of the individual standards at a concentration of 0.1 mg/mL were prepared by dissolving the compounds in methanol and were stored at 5 °C. Stock solutions of a mixture of all the standards in methanol, at concentrations of 100–1000 ng/mL, were prepared weekly. Working aqueous solutions of mixtures of all the standards were prepared daily by diluting the methanolic stock solution with water, to attain the required concentrations for calibration measurements. The water used in this work was purified with a Milli-Q water purification system from Millipore (Bedford, USA).

2.2. Sampling, sample pretreatment and preconcentration

Environmental water samples were collected in the autumn 2009 at several locations (The Vltava river in Prague, Uhlava river in Klatovy and Pilsen, all in the Czech Republic) and cover both densely inhabited areas, where urban wastewaters represent a significant input to the river water (Prague), and relatively clean areas (Klatovy, Pilsen). The river water samples, collected in precleaned amber glass bottles, were filtered with Mobile phase filtration apparatus (Supelco, Bellefonte, USA) through 0.45-μm Nylon filters (Millipore, Bedford, USA) to remove particulate and other suspended solid matter. The filtered samples were immediately preconcentrated by SPE using a Visiprep SPE Vacuum Manifold from Supelco (Bellefonte, USA). Three different types of SPE cartridges, all of 1 ml volume, packed with 100 mg of a C18 phase bonded to silica gel, were tested for their efficiency. These cartridges were a Sep-Pak Vac C18 with monomeric octadecyl-bonded silica, endcapped (Waters, Milford, USA), a polymeric Discovery DSC-18 and a monomeric Discovery DSC-18Lt, both with octadecyl-bonded silica, endcapped (Supelco, Bellefonte, USA). All the SPE columns used were first activated by rinsing with 5 mL of deionized water, 5 mL of methanol and 5 mL of deionized water, all at a flow rate of 5 ml/min. After the passage of the aqueous solution at a selected flow rate, the SPE column was rinsed with 10 mL of deionized water to remove highly polar components of the aqueous matrix and was dried by passage of nitrogen. The eluate was filtered again prior to its injection into the chromatographic system, through a 0.2 μm filter Spartan 13/0.2 RC (Sigma–Aldrich, St. Louis, USA).

2.3. Instrumentation and the experimental conditions

The HPLC experiments were perfomed using a Series 1200 Capillary Liquid Chromatograph with a Triple Quad LC/MS 6460 tandem mass spectrometer (Agilent Technologies, Waldbroon,

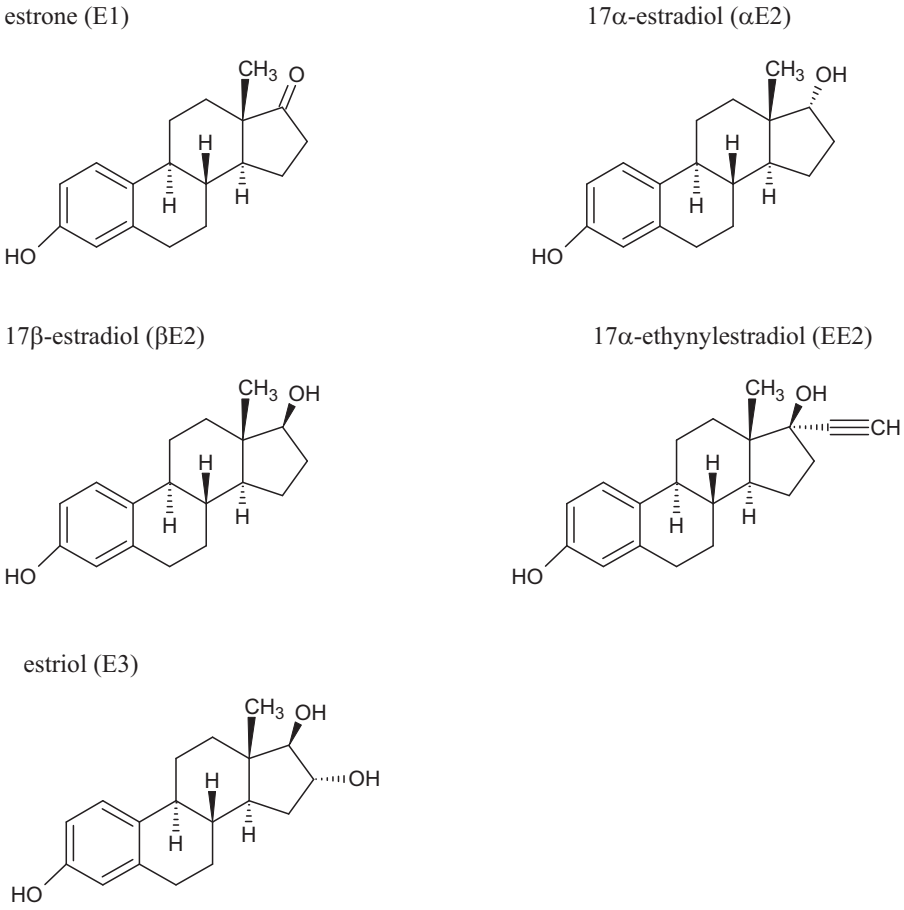

Fig. 1. Chemical structures of the estrogens studied.

Germany) and with an electrospray ionization interface, an automated injector, a column oven, a degasser and a quaternary pump. The chromatograms were recorded and evaluated by means of the MassHunter Workstation Acqusition (Agilent Technologies, Waldbroon, Germany). A Zorbax SB C18 capillary column (150 mm × 0.5 mm i.d., particle size 5 μm, Agilent Technologies, Waldbroon, Germany) was used. A PEEK inline microfilter including 0.5 μm frit (Supelco, Bellefonte, USA) protected the capillary column.

The chromatographic measurements employed both the isocratic and gradient elution. The mobile phases for isocratic elution were prepared by mixing acetonitrile and water (both containing 0.1% formic acid) at various volume ratios. The mobile phase flow rate was set at 18 μL/min. The gradient elution was realized with the binary mobile phase of acetonitrile containing 0.1% formic acid (A) and the aqueous solution of 0.1% formic acid (B), under various elution profiles at a flow rate of 18 μL/min. To protect the column against contamination, it was rinsed after approximately 20 analyses with pure acetonitrile for 1 h, to remove less polar substances.

The MS–MS measurements were performed in the MRM mode, using ESI in the positive mode. Nitrogen was used as the collision, nebulizing and desolvating gas.

3. Results and discussion

3.1. Optimization of the MS–MS conditions

All the instrumental MS–MS parameters have been optimized in a standard way. After injection of methanolic solutions of standards (100 ng/ml) into the ion source by the syringe pump, two most intense characteristic molecular fragments were selected by tuning the values of the fragmentor voltage (from 10 to 350 V) in MS2 SIM mode and the collision energy (from 10 to 250 V) in product ion mode. The resultant values are listed in Table 1. To maximize the signals in the MRM mode, the following parameters were tuned over the following ranges: capillary spray voltage, 1500–6000 V, the nebulizer pressure, 2–15 psi, the drying gas temperature, 250–350 °C, and the drying gas flow rate, 5–15 L/min. The optimimized ESI(+) conditions were: the capillary voltage, 5500 V,

Table 1
MRM conditions used for cLC–MS–MS determination of estrogens (ESI, positive mode).

Analyte	Precursor ion	Product ion	Fragmentor (V)	Collision energy (V)
E3	271.0	252.9	110	10
βE2	255.0	158.9	120	15
αE2	255.0	158.9	120	15
EE2	279.0	132.9	120	10
E1	271.0	252.9	110	10

the nebulizer pressure, 12 psi, the gas temperature, 350 °C, and the nitrogen flow-rate,10 L/min.

3.2. Optimization of cLC–MS–MS for determination and quantitation of target estrogens

The estrogens studied are medium polar or nonpolar acidic substances, whose log K_{ow} values lie within an interval of 2.45–3.13, in dependence on their structures [19]. Their HPLC–MS analyses mostly employ reversed-phase systems with C18 or C8 stationary phases and mobile phases containing methanol or acetonitrile organic modifiers and aqueous basic components (e.g., NH_3 and its salts), combined with the negative-mode ionization. However, application of a basic mobile phase to the silica-based stationary phases is often unsuitable because of the danger of silica gel dissolution, which is more pronounced with capillary columns than with standard HPLC ones. Therefore, at the beginning of the cLC study, ESI in the negative mode in combination with acidic mobile phase was tested but the signals attained for the test analytes were very low and noisy. This was one of the reasons for the selection of the acidic mobile phase in combination with the positive ESI mode.

To develop a miniaturized cLC procedure for determination of trace amounts of free estrogens in aqueous matrices, the Zorbax SB C18 capillary column was selected, as it is recommended for separations in solutions of very low pH values where it should provide a high stability, good reproducibility of the results and good peak symmetry, especially for acidic analytes. A binary mixture of acetonitrile and water, containing 0.1% formic acid in both the components, was used as the mobile phase. The system was optimized under the conditions of isocratic elution, following the effect of the acetonitrile content, within a range from 25 to 45 vol.%, on the retention, separation and the degree of ionization of all the five analytes in the positive ionization mode. The optimum composition of the mobile phase, from the points of view of a sufficient retention of the most polar analyte, estriol, an acceptable resolution of all the estrogens and a sufficient detection sensitivity, was found to be 38/62 (v/v) ACN/water mixture containing 0.1% formic acid. The time of analysis did not exceed 13 min. In contrast to the spiked deionized water, the retention of estriol, contained in a methanolic eluate after the preconcentration from environmental water samples, was insufficient under the isocratic conditions, due to the poor baseline in the beginning of the separation. A decrease in the acetonitrile content led to prolonging of the estriol retention, but the resolution of 17β- and 17α-estradiols became poorer. Therefore, gradient elution was applied. Various gradient profiles were tested and the optimized one started with 26% A which was increased to 38% within 3 min, maintained constant for 2 min, increased again to 42% within 3 min, maintained constant for 8 min, and finally returned to 26% within 1 min. All the compounds were eluted within 14 min and the total run time was 17 min. This gradient profile was applied to all the following analyses. An example of the separation under the optimized gradient elution conditions is depicted in Fig. 2.

3.3. SPE extraction

The SPE preconcentration was optimized considering the SPE sorbent type, the sample volume, the sample flow rate, the elution solvent and its flow rate. For each type of commercial SPE cartridge, the volume of deionized water (10–500 ml) was optimized, spiked with the analytes at a concentration of 50 ng/L. The spiked deionized water was applied to the cartridges at various flow rates (0.8–1.5 mL/min) and several flow rates were tested for the elution of the analytes (0.5–1.2 mL/min). The volume of the solvent necessary for complete elution of the analytes (0.5, 1.0, 1.5 and 2.0 mL) was determined for all the estrogens.

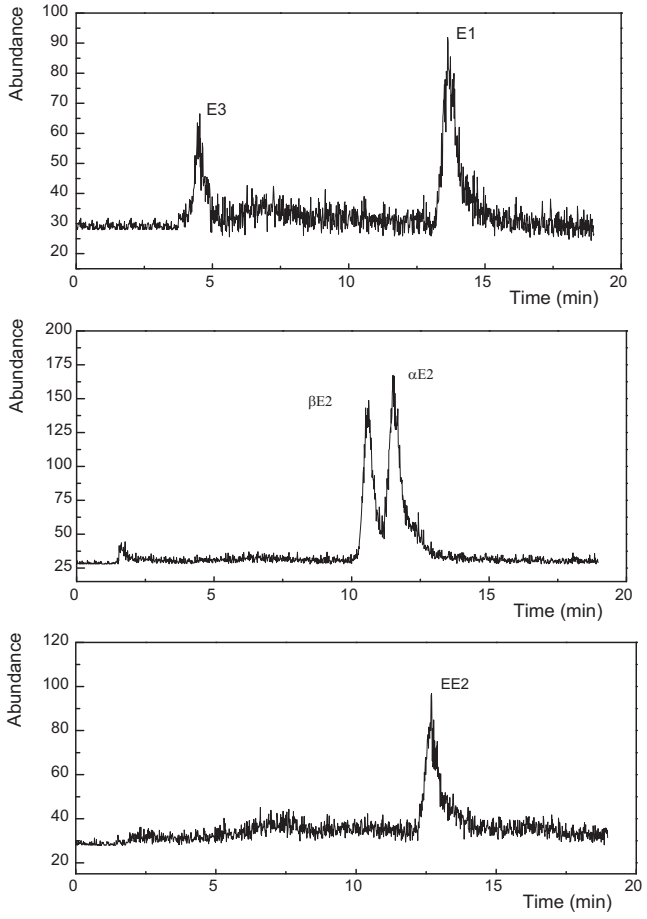

Fig. 2. MRM chromatograms of estrogens obtained for spiked river water (50 ng/L, Uhlava, Klatovy) under the optimized preconcentration and gradient elution conditions (see Sections 3.2 and 3.3, respectively).

The Sep-Pak C18 column exhibited very strong sorption of all the analytes, which were not eluted by the solvents tested (acetonitrile, methanol, ethyl acetate, acetone). To reduce the undesirable interactions of the analytes with the SPE packing, 0.5% of triethylamine was added to the elution solvent. Then, the analytes were partially eluted, but the yield did not exceed 30%. Therefore, this SPE column was no longer used and a new generation of SPE columns, namely, Discovery DSC-18 and DSC-18Lt, was tested. These cartridges permitted reversible sorption of all the analytes, the better results being provided by the monomeric-bound DSC-18Lt stationary phase, permitting a rapid elution of the analytes with pure methanol.

Under the optimized preconcentration conditions, namely, a Discovery DSC-18Lt column, 100 mL aqueous samples, a flow rate of 1 mL/min, elution with 0.5 mL of methanol at flow-rate of 1 mL/min, spiked deionized water was tested first. The results, obtained for three estrogen concentration levels, 25, 50 and 250 ng/L, are summarized in Table 2 (columns a–c). It can be seen that the yield generally amounts to 95–100%, with a very good repeatability (the RSD values up to 5.9%).

The Uhlava river water, coming from an unpolluted area, was selected as the environmental aqueous sample. The samples were collected close to the town of Klatovy, 50 km apart from the river spring. The preconcentrated, unspiked Uhlava river sample (Klatovy) was found to be free of the studied estrogens and was used as the environmental blank. Spiked at 25, 50 and 250 ng/L, Uhlava river water was preconcentrated and the extraction yield determined; the results are presented in Table 2 (columns d–f). The yields are in

Table 2
Recoveries and relative standard deviations (%RSD, $n = 3$) of the studied estrogens obtained for spiked deionized[a,b,c] and river water[d,e,f] (Uhlava, Klatovy), experimental conditions: samples eluted with 0.5 mL methanol, cLC analysis under the optimized gradient elution (for details, see Section 3.2).

Analyte	%[a]	RSD[a]	%[b]	RSD[b]	%[c]	RSD[c]	%[d]	RSD[d]	%[e]	RSD[e]	%[f]	RSD[f]
E3	95	5.8	98	3.5	97	4.0	95	7.2	96	5.3	96	6.1
βE2	98	5.2	100	3.0	100	4.0	98	6.5	100	2.2	100	3.3
αE2	97	5.9	100	3.0	99	4.0	98	5.1	99	2.5	99	3.4
EE2	98	5.4	99	3.3	98	4.2	97	5.3	98	3.2	98	3.9
E1	96	4.6	98	3.4	97	4.3	95	5.1	98	3.5	96	4.3

[a] 100 mL deionized water spiked at 250 ng/L.
[b] 100 mL deionized water spiked at 50 ng/L.
[c] 100 mL deionized water spiked at 25 ng/L.
[d] 100 mL river water spiked at 250 ng/L.
[e] 100 mL river water spiked at 50 ng/L.
[f] 100 mL river water spiked at 25 ng/L.

Table 3
Parameters of the calibration curves (standard deviations are in parentheses), limit of detection (LOD) and limit of quantitation (LOQ) obtained for spiked deionized water; for mobile phase gradient and optimized preconcentration conditions see Sections 3.2 and 3.3, respectively. Linearity range of analytes used: from LOQ to 500 ng/L.

Compound	Slope (L/ng a.u.)	Intercept (a.u.)	Correlation coefficient	LOD (ng/L)	LOQ (ng/L)
E3	10.21 (0.32)	−24.15 (1.87)	0.9998	4.7	17.3
βE2	43.55 (0.93)	−33.75 (3.28)	0.9996	3.9	7.3
αE2	60.42 (1.29)	−258.78 (9.09)	0.9985	3.0	6.1
EE2	37.86 (1.05)	−117.64 (5.62)	0.9998	3.4	12.3
E1	40.59 (2.33)	372.93 (32.75)	0.9989	5.4	15.8

the range of 95–100%, with a very good repeatability (the RSD values up to 6.5%, except for estriol). Comparing the results obtained for the deionized and river waters, it can be stated that the recoveries are higher than the values obtained with the commonly used SPE cartridges [11].

3.4. Quantitation

Under the optimized preconcentration and separation conditions, the calibration curves were measured for all the five estrogens in spiked distilled water and in spiked river water (Uhlava, Klatovy). The calibration curves were constructed in the concentration range from 10 to 500 ng/L, and the analytes were tested within a linearity range from LOQ of respective analyte to 500 ng/L. Each measurement of the peak area being carried out in triplicate. The results of the linear regression are listed in Table 3 (spiked deionized water) and Table 4 (spiked Uhlava river, Klatovy). The high correlation coefficients indicate a good agreement of the linear calibration with the results of the real sample analyses. Testing at a significance level of $1 - \alpha = 0.95$ demonstrates that the computed intercepts are statistically significantly different from zero.

The peak height—concentration dependences were treated by linear regression, to determine the limits of detection (LOD) and quantitation (LOQ), as the triple and ten-times the noise level, respectively. It can be seen that the LOD and LOQ values, obtained for spiked deionized water (Table 3) are lower than for the river water matrix (Table 4). The LOD and LOQ values obtained for spiked river water are not exceeding 6.8 and 25.0 ng/L, respectively. The highest LOD and LOQ values were always obtained for estriol, which is eluted first, and for estrone, whose retention time is the longest. The LOD and LOQ values are fully comparable with the published ones for HPLC on standard size columns and provide sufficient sensitivity for analyses of environmental aqueous samples [11].

3.5. Application to environmental samples

The optimized procedures for the analyte preconcentration and the subsequent analysis of the methanolic eluate using gradient elution were applied to six real aqueous samples collected between September 12 and December 14, 2009 at various sites, namely, Vltava river (Prague), the Botic stream (Prague), Uhlava river (Klatovy and Pilsen), wastewater entering and leaving the Imperial Island Prague WWTP. Each sample was preconcentrated in triplicate and analyzed. Only two samples yielded positive results for the presence of estrogens, namely, sample collected in the Vltava river (about 900 m beyond the Prague Central WWTP) and sample collected in the wastewater inlet stream into the WWTP on the Imperial Island in Prague. On the other hand, no estrogen was found in the outlet from the WWTP and in other Prague samples (the Botic stream). Samples from substantailly less inhabited areas (Uhlava river, Klatovy and Pilsen) also yielded negative results.

The MRM chromatogram of the Vltava river sample (Prague) is given in Fig. 3, indicating the presence of 13.2 ng/L (RSD 5.5%) of 17β-estradiol. The wastewater inlet into the WWTP contained all the studied estrogens, except for 17α-estradiol. The concentrations (ng/L) were 20.5 for estrone (RSD 8.4%), 21.4 for 17β-estradiol (RSD 7.2%), 100.7 for 17α-ethynylestradiol (RSD 4.4%) and 188.6 for estriol (RSD 6.8%); the sum of estrogens was 371.4 ng/L. The repeatability of the whole procedure, including both the extraction step and the subsequent analysis of the real sample, expressed in terms of RSD ($n = 9$), did not exceed 8.5%, which is a very good

Table 4
Parameters of the calibration curves (standard deviations are in parentheses), limit of detection (LOD) and limit of quantitation (LOQ) obtained for spiked river water (Uhlava, Klatovy), for mobile phase gradient and optimized preconcentration conditions see Sections 3.2 and 3.3, respectively. Linearity range of analytes used: from LOQ to 500 ng/L.

Compound	Slope (L/ng a.u.)	Intercept (a.u.)	Correlation coefficient	LOD (ng/L)	LOQ (ng/L)
E3	10.90 (0.53)	−37.26 (1.99)	0.9997	6.8	25.0
βE2	49.44 (1.99)	−57.89 (4.53)	0.9995	6.4	12.1
αE2	71.59 (2.65)	−348.46 (15.88)	0.9981	5.2	10.5
EE2	41.28 (2.24)	−140.99 (7.24)	0.9996	5.1	18.5
E1	43.77 (5.36)	435.38 (42.36)	0.9981	6.7	19.3

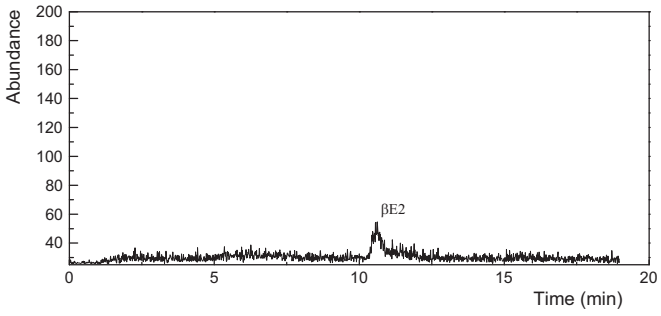

Fig. 3. A MRM chromatogram of the Vltava river sample obtained under the same conditions as those in Fig. 2. The peak corresponds to 17β-estradiol, its content is 13.2 ng/L.

result for an analysis of trace analytes in real samples. The RSD value depends on the character of the aqueous matrix and is higher for more polluted samples (compare the RSD values for 17β-estradiol obtained for the sample from the Vltava river and for that collected in the WWTP inlet). To test the suitability of the whole developed analytical procedure to the samples with more complex matrices, the wastewater samples from the inlet and outlet of the WWTP were spiked with all the studied estrogens at a level of 50 ng/L. The increased estrogen concentrations found in the spiked samples corresponded to the amount of the analytes added, demonstrating unchanged recoveries and the absence of any matrix-induced ion suppression during the detection.

The results obtained correspond to those published for classical HPLC analyses. The detection limits for HPLC–MS–MS analyses range from units of ng/L to tens of ng/L for free estrogens [11] and this fully agrees with the LOD values obtained in the present study. The estrogen contents in river and waste waters found by us are also comparable to the results published for Japanese and Spanish rivers and wastewaters or surface water of the Baltic Sea [11,20,24,29,33,38]. The negative results obtained for the outlet from the Prague WWTP document an efficient removal of estrogen pollutants from the water treated.

4. Conclusions

A new, miniaturized separation procedure, employing capillary liquid chromatography hyphenated with the MS–MS detection, has been developed for the determination of five important estrogen pollutants in natural waters, including the poorly separable isomeric pair of 17α- and 17β-estradiols. The optimized preconcentration and separation conditions permit the attainment of very low LOD and LOQ values, of the order of units to tens of ng/L, in aqueous matrices, permitting the determination of traces of the studied estrogens in samples of environmental waters. The use of new SPE materials permits a single-step preconcentration of all the estrogens with a high yield, close to 100%. These results, based on the advantage of a low solvent consumption in cLC and on the combination of the positive ionization ESI mode with a column friendly acidic mobile phase, predetermine this method for application in estrogen screening and series analyses.

Acknowledgements

Financial support from the Ministry of Education of the Czech Republic, the projects Centrum No. 1M06011, 285411 and from the long-term research plan of the Ministry of Education of the Czech Republic, No. MSM0021620857, are gratefully acknowledged. Supported by a grant from Norway through the Norwegian Financial Mechanism (Project CZ0116).

References

[1] T. Colborn, F.S.V. Saal, A.M. Soto, Environ. Health Perspect. 101 (1993) 378.
[2] C.R. Tyler, S. Jobling, J.P. Sumpter, Crit. Rev. Toxicol. 28 (1998) 319.
[3] S. Jobling, M. Nolan, C.R. Tyler, G. Brighty, J.P. Sumpter, Environ. Sci. Technol. 32 (1998) 2498.
[4] F. Eertmans, W. Dhooge, S. Stuyvaert, F. Comhaire, Toxicol. In Vitro 17 (2003) 515.
[5] R.M Sharpe, Phil. Trans. R. Soc. 365 (2010) 1697.
[6] J. Peknicova, V. Kyselova, D. Buckiova, M. Boubelik, Am. J. Reprod. Immunol. 47 (2002) 311.
[7] K. Dvorakova-Hortova, M. Honetschlagerova, E. Tesarova, J. Vasinova, M. Frolikova, Z. Bosakova, Folia Zool. 58 (2009) 75.
[8] H. Andersen, H. Siegrist, B. Halling-Sorensen, T. Ternes, Environ. Sci. Technol. 37 (2003) 4021.
[9] S. Reddy, C.R. Iden, B.J. Brownawell, Anal. Chem. 77 (2005) 7032.
[10] G.H. Panter, R.S. Thomson, N. Beresford, Chemosphere 38 (1999) 3579.
[11] V. Pacakova, L. Loukotkova, Z. Bosakova, K. Stulik, J. Sep. Sci. 32 (2009) 867.
[12] M.J.L. de Alda, D. Barcelo, Fresenius J. Anal. Chem. 371 (2001) 437.
[13] R.W. Giese, J. Chromatogr. A 1000 (2003) 401.
[14] M. Petrovic, M.D. Hernando, M.S. Diaz-Cruz, D. Barcelo, J. Chromatogr. A 1067 (2005) 1.
[15] M. Petrovic, E. Eljarrat, M.J.L. de Alda, D. Barcelo, J. Chromatogr. A 974 (2002) 23.
[16] P. Labadie, E.M. Hill, J. Chromatogr. A 1141 (2007) 174.
[17] M. Petrovic, S. Tavazzi, D. Barcelo, J. Chromatogr. A 971 (2002) 37.
[18] D. Matejicek, P. Houserova, V. Kuban, J. Chromatogr. A 1171 (2007) 80.
[19] K. Hajkova, J. Pulkrabova, J. Schurek, J. Hajslova, J. Poustka, M. Napravnikova, V. Kocourek, Anal. Bioanal. Chem. 387 (2007) 1351.
[20] T. Isobe, H. Shiraishi, M. Yasuda, A. Shinoda, H. Suzuki, M. Morita, J. Chromatogr. A 984 (2003) 195.
[21] A. Nieto, F. Borrull, E. Pocurull, R.M. Marce, J. Chromatogr. A 1213 (2008) 224.
[22] M. Pedrouzo, F. Borrull, E. Pocurull, R.M. Marce, Talanta 78 (2009) 1327.
[23] M.J.L. de Alda, D. Barcelo, J. Chromatogr. A 938 (2001) 145.
[24] M. Farre, M. Kuster, R. Brix, F. Rubio, M.J.L. de Alda, D. Barcelo, J. Chromatogr. A 1160 (2007) 166.
[25] Y. Suzuki, T. Maruyama, Water Res. 40 (2006) 1061.
[26] T.A. Ternes, H. Andersen, D. Gilberg, M. Bonerz, Anal. Chem. 74 (2002) 3498.
[27] J.D. Stuart, Adv. Chromatogr. 45 (2007) 245.
[28] A. Penalver, E. Pocurull, F. Borrull, R.M. Marce, J. Chromatogr. A 964 (2002) 153.
[29] K. Mitani, M. Fujioka, H. Kataoka, J. Chromatogr. A 1081 (2005) 218.
[30] J.F. Hsu, Y.C. Chang, T.H. Chen, L.C. Lin, P.C. Liao, J. Chromatogr. B 860 (2007) 49.
[31] Y. Watabe, T. Kubo, T. Nishikawa, T. Fujita, K. Kaya, K. Hosoya, J. Chromatogr. A 1120 (2006) 252.
[32] M.S. Diaz-Cruz, M.J.L. de Alda, R. Lopez, D. Barcelo, J. Mass. Spectrom. 38 (2003) 917.
[33] I.C. Beck, R. Bruhn, J. Gandrass, W. Ruck, J. Chromatogr. A 1090 (2005) 98.
[34] G. Morteani, P. Moller, A. Fuganti, T. Paces, Environ. Geochem. Health 28 (2006) 257.
[35] D. Matejicek, V. Kuban, J. Chromatogr. A 1192 (2009) 248.
[36] P. Sandra, in: 14th International Symposium on Separation Science, New Achievements in Chromatography, September 30 to October 3, Primošten, Croatia, Croatian Society of Chemical Engineers, 2008, p. 7.
[37] P. Coufal, Z. Bosakova, E. Tesarova, B. Kafkova, J. Suchankova, J. Barbe, J. Chromatogr. B 770 (2002) 183.
[38] M. Sole, M.J.L. de Alda, M. Castillo, C. Porte, K. Ladegaard-Pedersen, D. Barcelo, Environ. Sci. Technol. 34 (2000) 5076.